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SURVIVAL OF LARVAE AND NYMPHS OF *IXODES SCAPULARIS* SAY (ACARI: IXODIDAE) IN FOUR HABITATS IN MARYLAND

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Abstract.—Host-seeking and fed larvae and nymphs of the blacklegged tick, *Ixodes scapularis* Say, were placed in mesh packets and in vials in the leaf litter on the floor of mixed deciduous forest, Virginia pine-southern red oak forest, and white pine plantations with and without Nepal microstegium, *Microstegium vimineum* (Trinius) A. Camus. An introduced shade-tolerant grass, Nepal microstegium, is expanding its range northeastward into areas densely populated with *I. scapularis*. As determined by flag sampling, the density of host-seeking nymphs at the Virginia pine sites was much lower than in the other habitats. None of the four habitats appeared to be consistently more favorable or unfavorable for the survival of confined fed and unfed *I. scapularis* larvae and nymphs. More unfed nymphs survived in vials than in packets in Virginia pine and white pine with Nepal grass sites. Fed larvae and nymphs tended to survive the summer better than unfed ticks.

Key Words: blacklegged tick, immature stages, Nepal microstegium

In the United States, most cases of Lyme disease occur in an area from southern New England through the mid-Atlantic states (Spielman et al. 1985) with Maryland as the southernmost state with a significant Lyme disease problem. The blacklegged tick, *Ixodes scapularis* Say, the principal vector of the agent causing Lyme disease, is also involved in the transmission of the agents of babesiosis and human granulocytic ehrlichiosis (Spielman et al. 1985, Dumler and Bakken 1995). Off-host survival of fed and unfed ticks is affected by a variety of biotic and abiotic factors, such as natural enemies (e.g., predators, pathogens) and micrometeorological conditions (e.g., relative humidity) (Daniel and Dusbabek 1994). These factors seem to be associated with microhabit, but differences in survival of flat (unfed) *I. scapularis* nymphs may vary from one region to another independent of the type of microhabitat (Bertrand and Wilson 1997). Extreme temperatures and low relative humidities are harmful to *I. scapularis* (Stafford 1994, Vandyk et al. 1996). Ginsberg and Zhioua (1996) found that *I. scapularis* nymphs had a greater survival rate in deciduous forest compared to pine forest on Long Island, New York. Lord (1993) reported high mortality of unfed *I. scapularis* nymphs in New York. In Maryland, 64–70% of fed female *I. scapularis* placed in leaf litter in a deciduous forest survived to oviposit at least some eggs (Carroll 1996), but little is known about the survival of free-living larvae and nymphs.

In Maryland, mixed deciduous forests are a common natural habitat, varying in composition of dominant plant species according to soil type, drainage and other factors. Blacklegged ticks are typically found in these deciduous woodlands in Maryland (Schmidtmann et al. 1994, Carroll and Kramer 2001). Often, on well-drained soils practically contiguous with the deciduous forests there are stands of Virginia pine, *Pinus virginiana* Miller, and southern red oak, *Quercus falcata* Michaux. In central Maryland, white pine, *P. strobus* L., does not occur naturally (Elias 1980) but is sometimes planted as ornamental or in monocultures as a form of reforestation. Blacklegged ticks also occur in the pine-dominated habitats. White-tailed deer, *Odocoileus virginianus* (Zimmermann), and other important hosts of *I. scapularis* readily move among all three habitats. Nepal microstegium, *Microstegium vimineum* (Trinius) A. Camus (Poaceae), is a shade-tolerant, non-native species of annual (sometimes perennial) grass. Since being introduced into Tennessee over 80 years ago, *M. vimineum* has attained the status of an invasive weed in the U. S., while expanding its range northeastward into Maryland, New York, and New England (Hunt and Zaremba 1992, Redman 1995, Ehrenfeld 1999). Nepal microstegium has experienced explosive distributional growth in Maryland since the 1980s (Redman 1995). In areas around Loch Raven Reservoir, Baltimore County, where there are extensive plantings of white pine, *M. vimineum* is the dominant understory plant species, covering considerable surface area and attaining heights of $\approx$0.3 m. *Ixodes scapularis* is abundant in these white pine and adjacent deciduous woodlands (Carroll, unpublished data). Nepal grass forms sprawling colonies that might provide shaded, humid refugia for larvae and nymphs of *I. scapularis* and enhance their survival during late spring and summer. The grass dies and collapses in the fall. The purpose of this study was to obtain preliminary information on the survival of fed and unfed *I. scapularis* nymphs in the deciduous forest, Virginia pine-oak forest, white pine plantations with Nepal grass, and white pine plantations without Nepal grass.

Materials and Methods

The deciduous forest study sites were located at Loch Raven Reservoir, Baltimore County, Maryland, as were the white pine plantings with and without Nepal grass. The Virginia pine-southern red oak forests were located in the U. S. Fish and Wildlife Service's Patuxent North Tract in adjoining Anne Arundel County. The deciduous forest was dominated by tulip tree, *Liriodendron tulipifera* L., red maple, *Acer rubrum* L., hickory, *Carya* sp., and black cherry, *Prunus serotina* Ehrhart. Understory vegetation was generally sparse in the deciduous woods, with some ferns and Nepal grass present. Leaf litter in these deciduous forests was notably shallow, and in some places virtually gone by mid–late summer, perhaps due to the abundance of earthworms that were observed. In contrast, leaf litter in the Virginia pine-southern red oak forests remained $\approx$10 cm deep. Few other species of trees were present in these woods, and blueberries, *Vaccinium* sp., and greenbrier, *Smilax* sp., were common in the understory. The white pine plantations were virtually monocultures, with ferns and Nepal grass being the principal understory species.

For each of the four types of habitat, three sites ($\geq$0.8 km apart) were selected (Fig. 1). Each site was sampled for the presence of *I. scapularis* by flagging with a 0.5 by 0.5 m flannel cloth (crib cloth containing a rubber laminate) 5 times for 30 sec while walking slowly. At each of the three sites, confined ticks were placed in the leaf litter (where loose litter adjoined compacted litter or soil) at each of three locations, with the exception of unfed larvae, which were placed at one randomly selected location at each site in 1999. At each site each group of ticks was placed $\geq$10 m from the nearest group of confined ticks. The location of each group was marked with a flag. Unfed and fed larvae and nymphs of *I. scapularis* were placed at these locations in 1999 and

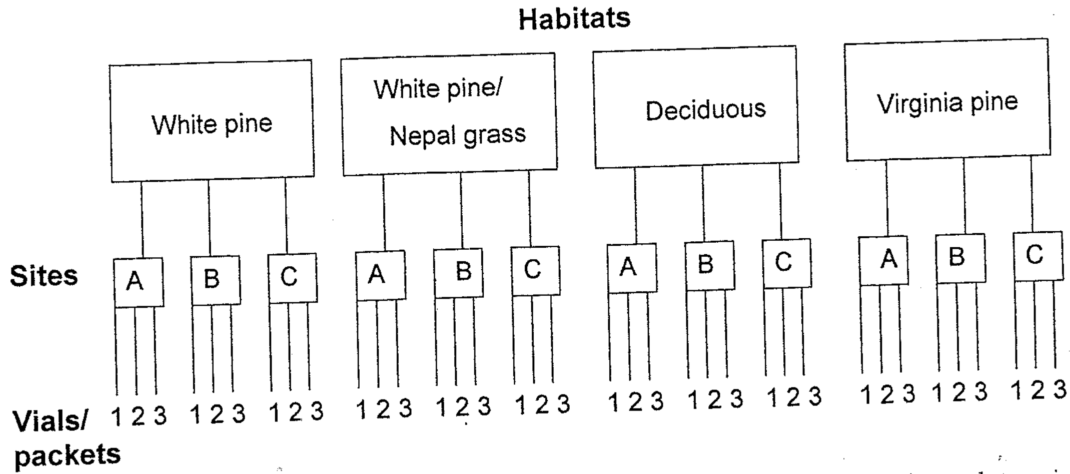

Fig. 1. For each of 4 types of habitat, unfed and fed larvae and nymphs confined in nylon packets or in vials were placed in leaf litter at 3 sites (designated here as A, B and C). At each site the packets and vials were placed at 3 marked locations (designated here as 1, 2 and 3) ≥10 m apart.

2000 when free-living ticks were in the same stage of development. Unfed larvae and nymphs were collected from the field, placed in plastic snap-cap vials and maintained at 24–25° C, R. H. ≈97%, and natural photoperiod. Fed larvae and nymphs were allowed to engorge on white rats in accordance with an approved USDA, ARS, Beltsville Agricultural Research Center, IACUC protocol. Within 4 d after dropping from a rat, the fed larvae and nymphs were placed in nylon packets to be distributed at the study sites. The packets were of 82 by 82 mesh per cm^2 folded once to form a rather flat ≈2 by 5 cm rectangle sealed on two sides by adhesive applied with a glue gun. Once the ticks were placed inside the packet, the fourth side was folded over and closed as securely as possible with a bulldog clip. In 1999, 10 unfed larvae, 5 fed larvae, 6 unfed nymphs, and 3 fed nymphs were placed in each packet. The following year 8 unfed larvae, 6 fed larvae, 3 unfed nymphs, and 3 fed nymphs were placed in each packet. In 1999, unfed nymphs (6 per vial) were placed in plastic snap-cap vials (3 dram) with a 0.8 cm diameter hole in the cap and nylon cloth covering the mouth of the vial. Except in 1999, when one packet containing unfed larvae was placed at one of the three locations (chosen randomly) at each of the three sites for each habitat, one packet or vial containing ticks was distributed to each location at each site. Nymphs were placed out in late spring to early summer, and larvae in August. Unfed ticks were removed from the field and checked for mortality near the end of the natural activity periods for larvae and nymphs (late August to September). Fed ticks were checked after the time that individuals of the same stage in nature should have molted into the next life stage. Standard errors were calculated for surviving ticks by habitat. Mean numbers of ticks in surviving vials and packets were compared using Student's t-test.

Results

At least one nymph or adult of *I. scapularis* was found by flagging at all but one of the study sites where ticks were placed (Table 1). However, the Virginia pine-oak habitat contained few *I. scapularis*. The summer of 1999 and the fall of 2000 were unusually dry, whereas the summer of 2000 was wet. In 1999, unfed larvae survived poorly in all habitats (≤43%), but especially so in the deciduous forest (7%), which had significantly fewer ($P = 0.004$) survivors than the Virginia pine-southern red oak

Table 1. Numbers of *I. scapularis* nymphs and adults captured by flagging 5 times for 30 sec while walking slowly at each study site just before the first packets of ticks were placed in the leaf litter.

	Virginia Pine		Deciduous		White Pine Open		White Pine Grass	
	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults
Site 1	1	0	9	0	7	0	7	1
Site 2	0	0	7	0	23	0	10	0
Site 3	0	1	6	0	10	0	1	0

sites (Table 2). Survival of unfed larvae was even worse in 2000, with <10% surviving. However, despite overall low levels of survival of unfed larvae in 2000, larval survival was somewhat greater ($P = 0.0496$) in the white pine with Nepal grass sites than the deciduous forest and white pine without Nepal grass sites. In 1999 fewer unfed nymphs in packets survived at the Virginia pine sites than at the deciduous forest sites ($P = 0.014$) (Table 3). At the Virginia pine and white pine with Nepal grass sites, significantly more unfed nymphs survived in vials than in packets ($P < 0.05$). Very few unfed nymphs (≤2%) survived in 2000. In general, substantial proportions of fed *I. scapularis* larvae and nymphs molted to the next stage and no differences in survival of fed ticks among the habitats were detected. In 1999, >72% of fed larvae in all four habitats survived to become nymphs, but in 2000 only about half (36–59%) survived. Most fed nymphs in all habitats survived to become adults, with >62% surviving in 1999 and >83% surviving in 2000.

Discussion

Based on flag sampling of nymphs and adults, the Virginia pine-southern red oak sites in this study appeared to support very few *I. scapularis*. Because Virginia pine habitats are often contiguous with deciduous forests in Maryland, and share deer and other hosts of *I. scapularis,* further comparative sampling of *I. scapularis* in these these habitats is warranted. Ginsberg and Zhioua (1996) also found lower densities of *I. scapularis* in pine (mostly pitch pine, *P. rigida* Miller) woods than deciduous thickets. In New Jersey Schulze et al. (1998) found *I. scapularis* nymphs much more numerous in pitch pine habitats than in three other habitats not dominated by pines at one study area. At a second study area where white pine was the dominant species, num-

Table 2. Numbers of unfed and fed larvae of *I. scapularis* surviving in each of the 4 habitats. Means per location on following line; percentage of ticks surviving in each habitat in parentheses.

	Virginia Pine	Deciduous	White Pine Open	White Pine Grass
Unfed Larvae				
1999	13/30[a] (43%)	2/30 (7%)	9/30 (30%)	10/30 (33%)
	4.3 ± 0.9	0.7 ± 0.7	3.0 ± 0.6	3.3 ± 1.9
2000	3/90 (3%)	2/90 (2%)	2/90 (2%)	8/90 (9%)
	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.9 ± 0.3
Fed Larvae				
1999	15/20 (75%)	33/40 (83%)	18/25 (72%)	22/30 (77%)
	3.8 ± 0.5	4.7 ± 0.2	3.6 ± 0.5	3.8 ± 0.5
2000	32/54 (59%)	23/42 (55%)	15/42 (36%)	23/48 (48%)
	3.6 ± 0.7	3.3 ± 0.7	2.1 ± 0.5	2.9 ± 0.4

[a] Denominators (total number of ticks) vary because not all packets containing ticks were recovered. Some recovered packets were not intact, and all ticks had escaped.

Table 3. Numbers of unfed and fed nymphs of *I. scapularis* surviving in each of the 4 habitats. Means per location on following line; percentage of ticks surviving in each habitat in parentheses.

	Virginia Pine	Deciduous	White Pine Open	White Pine Grass
Unfed Nymphs				
In Packets				
1999	11/54[a] (20%)	26/48 (54%)	19/54 (35%)	9/42 (21%)
	1.2 ± 0.5	3.3 ± 0.5	2.1 ± 0.6	1.3 ± 0.7
2000	1/54 (2%)	0/48 (0%)	1/54 (2%)	0/48 (0%)
	0.1 ± 0.1	0	0.1 ± 0.1	0
In Vials				
1999	29/54 (54%)	23/48 (48%)	20/48 (42%)	25/42 (60%)
	3.2 ± 0.6	2.9 ± 0.6	2.5 ± 0.6	3.6 ± 0.7
Fed Nymphs				
1999	9/9 (100%)	9/12 (75%)	13/21 (62%)	10/12 (83%)
	3.0 ± 0	2.3 ± 0.5	1.9 ± 0.3	2.5 ± 0.3
2000	21/24 (88%)	20/24 (83%)	8/9 (89%)	16/18 (89%)
	2.6 ± 0.2	2.5 ± 0.2	2.7 ± 0.3	2.7 ± 0.2

[a] Denominators (total numbers of ticks) vary because not all packets or vials containing ticks were recovered. Some recovered packets and vials were not intact, and ticks had escaped.

bers of *I. scapularis* nymphs were not higher than in three other habitats not dominated by pines (Schulze et al. 1998).

In the present study, Virginia pine sites did not stand out as inhospitable to the survival of *I. scapularis* larvae and nymphs, rather survival of fed *I. scapularis* larvae and nymphs did not appear to vary consistently among the four types of habitat. As the summers progressed, the leaf litter in the deciduous forests steadily diminished to the extent that in a few instances packets were found exposed on bare soil, at the exact locations where they had been covered with fallen leaves when originally placed. This unusual (in the author's experience in Maryland deciduous forests) phenomenon may account for the comparatively poor survival of flat nymphs in packets in deciduous woods in the drought year 1999. Stafford (1994) found that nymphs of *I. scapularis* require elevated relative humidities for extended survival. Lord (1993) reported high mortality (90% in 45 d) of unfed *I. scapularis* nymphs confined in mesh packets (10 by 20 cm) partly buried in leaf litter and soil in deciduous woods in southern New York. With totally exposed packets subject to more desiccating conditions, it is not surprising that many ticks died. In the following year, which experienced regular summer rains, virtually all unfed nymphs in all habitats died. Only in 2000, when survival of unfed larvae was extremely low at all sites, was there any indication that Nepal grass colonies might be more favorable for survival of either life stage of *I. scapluris* than the open white pine woods. In general, flat nymphs and larvae survived better in 1999 than in 2000 when rainfall was plentiful. Flooding or prolonged submersion of the confined ticks may have caused mortality directly or created conditions favoring fungal or perhaps even bacterial pathogens harmful to the ticks. Using larger packets or cages which extend upward out of the litter would give mobile ticks the opportunity to move to more favorable microenvironments when conditions change (Yuval and Spielman 1990, Lord 1993). However, in the case of Lord (1993), unfed nymphs survived as poorly (<10%) as the unfed nymphs did in this study in 2000. In 1999, 20–54% of unfed nymphs in all four habitats survived the summer. Host-seeking nymphs of *I. scapularis* can disperse ≥5 m, thereby avoiding some deleterious situations (Carroll and Schmidtmann 1996). Although fed larvae and nymphs lack the mobility of their unfed counterparts, much higher percentages of fed than unfed ticks survived in both years.

In the late summer of 1999, an unknown person removed all the flags marking the positions of the tick packets from one open white pine site and a nearby white pine site with Nepal grass. Despite written descriptions of the locations of the packets, some were not found. A few other packets, perhaps removed by rodents or birds, were never recovered. Some packets, which were recovered, were found to have holes through which all the ticks escaped. These losses may have reduced some of the anticipated discriminating power of the study, but enough packets were recovered to detect any gross differences and some more subtle differences in tick survival among the habitats.

In conclusion, even though the study took place during a unusually dry summer and a wet summer, none of the habitats appeared obviously more favorable or unfavorable for survival of confined fed and unfed larvae and nymphs of *I. scapularis*. Further comparative sampling of host-seeking *I. scapularis* in Virginia pine-southern red oak and other Maryland habitats is needed.

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